Plumage reflectance is not affected by preen wax composition in red knots Calidris canutus

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It has recently been shown that sandpipers (Scolopacidae) abruptly switch the chemical composition of their preen gland secretions from mono- to diester waxes just before the period of courtship. The timing and context of the shift suggested that diesters could provide a visible quality signal during mate choice. We used captive red knots *Calidris canutus* to test whether mono- and diester preen waxes affect the light reflectance ("colour") of the plumage. We also determined light absorbance spectra of the two wax types. The reflectance of breast feathers of the breeding plumage was measured with spectrophotometry when birds secreted monoesters and six weeks later when they secreted diester preen waxes. Light reflectance was also measured after removing the mono- and diester waxes from the plumage with a solvent. The results show that: (1) diester preen waxes absorb more light, especially ultraviolet (UV), than monoester preen waxes, but that (2) the compositional shift in the preen waxes did not change plumage reflectance and, (3) the removal of preen waxes did not change the reflectance of the plumage within the light spectrum assumed visible to birds (320–700 nm). This is not consistent with the idea that compositional shifts in the preen waxes of red knots have a visual function.

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Plumage colouration of birds has an important signalling function in the context of mate choice and during conflicts (Andersson 1994). Sexual selection through mate choice favours brightly coloured plumages (e.g. Hill 1991). The reliability of bright plumage traits as quality signals is related to the potential costs associated with the acquisition and maintenance of a conspicuous plumage (Zahavi 1975). Conspicuous plumages may also attract predators (Butcher and Rowher 1989). The trade-off between impressing potential mates and attracting predators is generally believed to be the underlying mechanism for many bird species to carry conspicuous plumage during the breeding season only.

Complete replacement of contour feathers requires a moult of several weeks and is therefore not a quick and flexible mechanism to adjust plumage colouration to accommodate abruptly changing needs. To avoid costly and time consuming moult, male rock ptarmigans *Lagopus mutus* soil their conspicuous white breeding plumage with dirt as soon as mating has taken place to increase crypsis (Montgomerie et al. 2001). The birds clean off the dirt when mating opportunities become available again, e.g. after a clutch is lost. Similar behaviour has been observed in bearded vultures *Gypaetus barbatus* that actively stain their plumages with orange soil containing iron oxide (Negro et al. 1999).

Greater hornbills *Buceros bucornis* use their preen gland secretions to yellow plumage areas that are used for signalling during threat displays (Elder 1954, Del Hoyo et al. 2001). Piersma et al. (1999) suggested that the diester preen waxes produced just before and at the beginning of the breeding season by red knots *Calidris canutus* may also have a cosmetic function. For most of the year the uropygial gland secretions of red knots consist of monoesters. A more detailed study of 19 sandpiper species (Reneerkens et al. 2002) showed that shifts from mono- to diester preen waxes are common to all of these species and that diester preen waxes are continuously secreted throughout the period of incubation by the incubating sex only. In red knots both of the sexes incubate and both sexes secrete diester preen waxes during incubation (Reneerkens et al. 2002). Therefore, the secretion of diesters is better correlated with incubation than with courtship and display.

For the diester preen waxes to function as avian cosmetics, shifts in preen wax composition should bring about a visual change of the plumage as seen by the birds themselves. We could not see a difference in plumage colouration of red knots with a shift in preen wax composition. However, most diurnal birds can detect wavelengths in the UV portion of the spectrum down to 320 nm, which are invisible to humans (Burkhardt 1989, Bowmaker et al. 1997). Therefore, a possible change in plumage appearance in red knots could remain unnoticed by human investigators (Bennett et al. 1994). In addition, red knots and other sandpipers breed in (sub) arctic regions with long days of sunshine and snow that increases the UV radiation load (Caldwell et al. 1980). Absorption of UV light by diester preen wax could protect feathers against potential damage and also affect reflectance of the plumage. We used spectrophotometry to determine both light absorption of mono- and diester preen waxes and the effect of both preen waxes on the reflectance of the contour feathers of red knots in breeding plumage.

Materials and methods

The birds and their preen waxes

We used six captive knots kept in two separate outdoor aviaries. Captive red knots exposed to the Dutch photoperiodic regime undergo semi-natural annual cycles in body mass and moult (Piersma and Ramenofsky 1998). Contour feather moult from a grey winter plumage to a rusty red breeding plumage takes place between March and May. The captive red knots also show annual cycles in preen wax composition (J. Reneerkens and T. Piersma unpubl. data). Preen wax was sampled twice a week by massaging the feathered nipple of the preen gland with a cotton bud. The wax was dissolved in ethyl acetate to a concentration of 1 mg wax/ml. The composition of the intact waxes was determined with capillary gas chromatography as described elsewhere by Dekker et al. (2000). The preen wax composition of all individuals was analysed every week so that we were able to compare reflectance of the breeding plumages of red knots before and after the chemical shift from mono- to diester preen wax.

Effect of presence of different preen waxes

The absorption spectra (190–900 nm) of mono- and diester preen waxes were obtained using a Uvikon 940 spectrophotometer. As a reference we used pure ethyl acetate. Absorbance spectra of six monoester and four diester samples were measured. The analysis of the absorption spectra was restricted to wavelengths above 290 nm only, as solar ultraviolet radiation shorter than 290 nm is absorbed in the high atmosphere by ozone and oxygen and absent in the earth's atmosphere (Gates 1966).

Reflection spectra of the red knots' plumages were measured on 9 May 2002 when the birds were secreting monoester preen waxes, and on 24 June 2002 when they were secreting diester preen waxes. Preen waxes on feathers were always the same as the waxes harvested from the uropygial gland (M. Dekker unpubl. data). Therefore, we are confident that the wax on the plumage had the same composition as the wax sampled from the uropygial gland, especially as the shift to diester preen waxes took place 1–3 weeks before the second reflectance measurements. As prealternate moult of contour feathers was completed before measurements were performed, possible differences in reflectance of birds between dates could not be attributed to replacement of feathers. Both on 9 May and on 24 June 2002 reflection spectra of the rusty red breeding plumage of the birds were measured before and after removal of preen waxes from the breast feathers. We removed waxes with a solvent (ethyl acetate) on cotton wool after the initial reflectance measurements. The cotton wool often turned yellowish during this treatment. This colour was similar to a cotton bud after massaging the preen gland nipple. Several minutes after this treatment we checked whether the solvent had completely evaporated by the absence of the smell of ethyl acetate before measuring the reflectance the second time.

To check whether with this treatment all of the preen waxes were effectively removed from the breast feathers, three red knots that were not used in the experiment underwent this treatment twice in a row. All waxes were extracted with ethyl acetate from the cotton wool and analysed by gas chromatography conform the analysis of the preen wax samples. We expected to find waxes after the first treatment but not after the second treatment. The gas chromatograms showed that after both treatments many more unidentified hydrophobic compounds were extracted from the plumage than preen waxes. Only a small fraction (less than a percent) of all removed compounds consisted of preen waxes.

Reflectance of the breast feathers was measured with an AVS-USB-2000 (Avantes, Eerbeek, The Netherlands) spectrophotometer, with illumination by an Avantes DH-2000 Halogen-Deuterium light source, both connected to the measuring probe by a bifurcated fibre optics cable. The measuring probe was fitted with a round plastic tube (6.35 mm inner diameter) to exclude ambient light and to keep the distance between the probe and the feathers constant. During the measurements the probe was held at a right angle to the plumage, i.e. both illumination and recording were at 90° to the feathers. Of each bird the reflectance of ten haphazardly selected spots on the breast were measured. Reflectance was expressed relative to a WS-2 white reflection standard (Avantes).

Statistical analyses

We used principal component analysis (PCA) to reduce the information contained in the absorbance and reflectance curves, which consist of many correlated reflectance values, into a few variables adequately describing the existing variation among curves (Endler 1990). We performed a PCA without factor rotation on correlation matrices of 22 absorbance values separated by 20 nm intervals between 290 nm and 710 nm and of 20 reflectance values of each curve between 320 and 700 nm. We only extracted principal components that had eigenvalues greater than 1. Non-parametric statistics (Mann-Whitney U test) were used to analyse absorbance measurements because the principal component was not normally distributed.

Spectral analyses of the reflectance curves of the red knots' plumages were restricted to 320–700 nm, the spectral range that is likely to be visible to most birds (Maier 1994). Raw spectra were smoothed by calculating a running mean over a 20 nm interval. Subsequently, the ten smoothed reflectance curves obtained for each bird were averaged.

Results

The first principal component (PC1) of the PCA explained 96% of the variation in the absorbance curves. The loadings of PC1 were all positive and higher than 0.90 except for 290 nm which had a loading of 0.61. Absorption spectra of preen wax samples revealed that diester waxes absorbed light better than monoester waxes (Mann-Whitney U, $Z = -2.56$, $P < 0.0001$), especially in the UV (Fig. 1).

The difference in light absorption did not result in a change of plumage reflectance with the shift from mono- to diesters in June (Fig. 2). Reflection spectra of the breeding plumages of red knots are typical of red or chestnut-coloured feathers (Burkhardt 1989). There is no reflectance peak in the UV band (between 320–400 nm), and reflectance gradually rises with wavelength (Fig. 2). The highest average percent reflectance occurs in the long wavelength portion of the light spectrum.

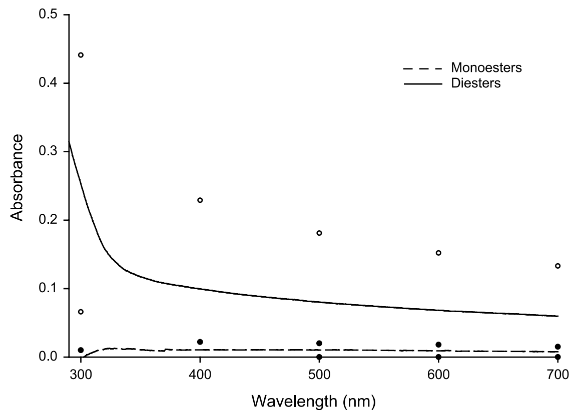

Fig. 1. Absorption spectra of mono- (dashed line) and diester (solid line) preen waxes. Six samples of monoesters were analysed and four samples of diesters. Dots indicate 95% confidence intervals at 100 nm increments for monoesters (black dots) and diesters (white dots).

The PCA adequately described the variation among measured reflectance curves. PC1 explained 92.0% and the PC2 explained 7.0% of the total variation in reflectance values among reflectance curves. PC1 had strong positive loadings (between 0.86 and 1.00) of all wavelengths along the whole 320–700 nm range, which indicated that PC1 was correlated with achromatic brightness of the plumage. This was confirmed by a strong correlation between PC1 and average percent reflectance between 320–700 nm ($r = 0.994$, $P < 0.0005$, $n = 24$). When using PCA to summarise reflectance curves the extracted PC2 is typically associated with variation in the shape of the curves, which may be visually perceived as variation in hue or chroma (Endler 1990). PC2 showed low to moderate negative loadings (between -0.01 and -0.31) from wavelengths between 320–520 nm, and low to moderate positive loadings (between 0.04 and 0.49) from wavelengths between 540–700 nm. Thus, the plumages of birds with high PC2 scores have relatively lower reflectance between 320–520 nm and relatively higher reflectance between 540–700 nm.

A change of preen wax composition from mono- to diesters did not result in a statistically significant change in PC1 (Fig. 2A; paired t-test $df = 5$, $t = -0.19$, $P = 0.86$). It did change the shape of the reflectance curves as indicated by the statistically significant change in PC2 (paired t-test $df = 5$, $t = 6.12$, $P = 0.002$), although the magnitude of the effect was small (Fig. 2). The significant difference in PC2 between May and June persisted for feathers of which preen waxes were removed (paired t-test $df = 5$, $t = 4.77$, $P = 0.005$), indicating that the small change in shape of the reflectance curves was not caused by a change in wax composition from May to June.

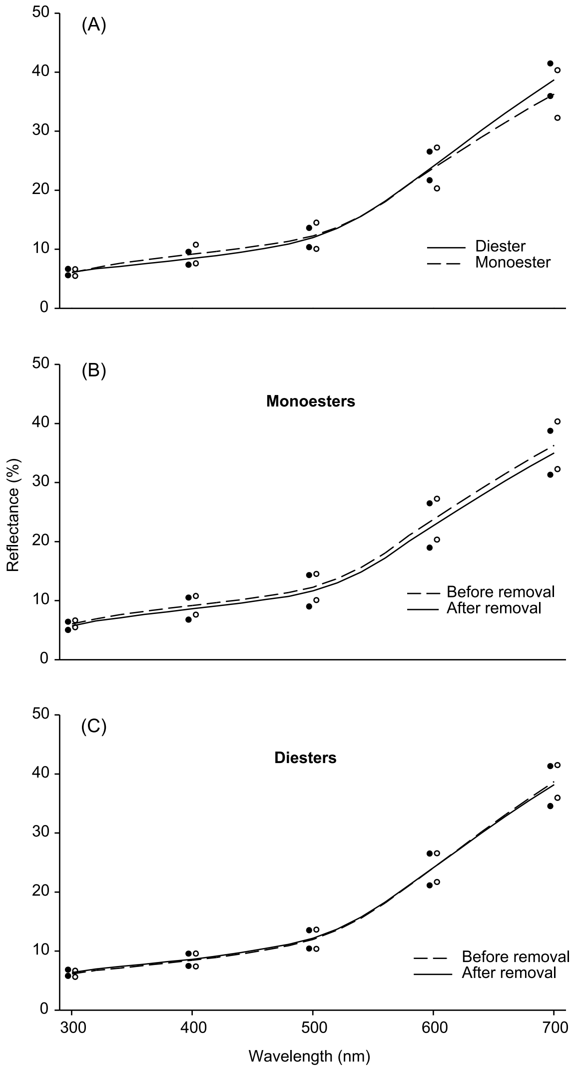

Fig. 2. Pairwise comparison of reflectance of breasts of six red knots in breeding plumage with monoester and diester preen waxes (A), before and after removal of monoester preen waxes (B), and before and after removal of diester preen waxes (C). Dots indicate 95% confidence intervals at 100 nm increments (staggered for clarity reasons) of reflectance curves of birds with diester (black dots) and monoester (white dots) preen waxes in (A) and of birds of which waxes have been removed from the plumage (black dots) and of birds before preen wax removal (white dots) in (B) and (C).

Removal of monoester (paired t-test, df = 5, PC1: t = 0.72, P = 0.50; PC2: t = −0.44, P = 0.68; Fig. 2B), or diester preen waxes (PC1: t = 0.07, P = 0.95; PC2: t = 1.68, P = 0.15; Fig. 2C) did not affect reflectance. This again suggests that the differences in the shape of the reflectance curves, as indicated by the significant change in PC2 between May or June, are probably not caused by the change in preen wax composition between those dates.

Discussion

Although diesters absorb more light than monoesters, this did not affect reflectance of plumage with a coating of mono- or diester preen wax. Light reflectance of the red knots' plumages is relatively little for wavelengths below 500 nm, even after preen waxes were removed, suggesting that feathers themselves absorb much light in this part of the light spectrum. This would imply that with a change from a coating of mono- to diester waxes on the feathers, less light of short wavelengths such as UV is absorbed by the feathers and more by the waxes without a net effect on plumage reflectance.

Plumage brightness of the red knots did not differ between May (monoesters) and June (diesters), but a small change in the shape of the reflectance curves between May and June was observed (Fig. 2). We do not know if the magnitude of the effect is large enough to be distinguishable to red knots. Because feathers of which preen waxes were removed showed the same difference between May and June as feathers with preen waxes of different composition, the shift in the shape of the reflectance curves is unlikely to be caused by the shift in preen wax composition. The reflection spectra of the feathers themselves did change slightly, probably as a result of feather wear (Örnborg et al. 2002).

Because we removed a large amount of hydrophobic compounds (several mg) from the breast feathers with the ethyl acetate, we believe that the lack of a change in visual appearance of red breast feathers of red knots indicates that the colour of feathers is mainly caused by the feather pigments. The layer of waxes and other compounds on feathers is probably too thin to cause a detectable change in light reflectance. This may also explain the discrepancy between the yellowish colour of smears from the preen gland and the lack of change in visual appearance of the plumage after preen wax removal. This is not consistent with the idea that colour changes during preen wax shifts play a role in the visual communication in red knots (Piersma et al. 1999), but there is still the possibility that the different preen wax compositions induce a different shine on the plumage (cf. the effect of shoe polish on leather).

The increased absorbance of light, and especially UV, by diesters could be functional. Diesters may better protect feathers against harmful UV radiation than monoesters, but this idea needs further testing. Although red knots are subjected to 24 hrs of sunlight on their breeding grounds and the presence of snow may increase the UV radiation load, arctic regions generally have a lower UV radiation load than temperate or tropical wintering grounds of red knots (Caldwell et al. 1980). It is also unclear why diester preen waxes are only secreted by incubating individuals (Reneerkens et al. 2002). Currently, two other hypotheses are being investigated: (1) less volatile diester preen waxes diminish the scent of incubating birds and hence increase 'olfactory crypsis' (Reneerkens et al. 2002), and (2) diester preen waxes protect birds' feathers more efficiently than monoesters against feather degrading bacteria, fungi and lice during periods during which they spend much time on the nest.

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